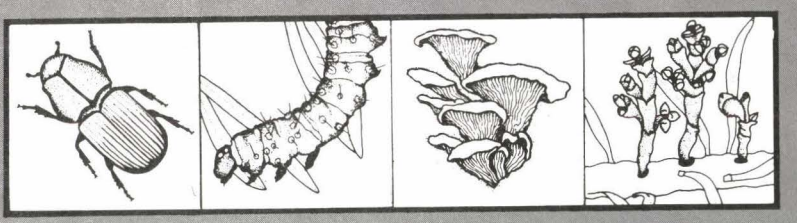
SB761
U7N6
88-10

Forest Pest Management



Report 88-10

3450
June 1988

# FUNGAL COLONIZATION OF STYROBLOCK CONTAINERS PLUM CREEK NURSERY, PABLO, MONTANA

*R. L. James, Plant Pathologist*

*and*

*C. J. Gilligan, Biological Technician*

## ABSTRACT

An evaluation of the efficacy of steam treatment on reducing levels of *Fusarium*, *Cylindrocarpon*, and *Trichoderma* within styroblock containers was conducted at the Plum Creek Nursery in Pablo, Montana. Although levels of both *Fusarium* and *Cylindrocarpon* were significantly reduced by cleaning, relatively high populations of both these fungi persisted after cleaning. *Fusarium* propagules were mostly concentrated at the bottom of styroblock cells as were those of *Cylindrocarpon*. Levels of *Cylindrocarpon* were much higher than either *Fusarium* or *Trichoderma*. *Trichoderma* levels were not significantly affected by the cleaning treatment. Seedling height was not correlated with extent of styroblock cell colonization by either *Fusarium* or *Cylindrocarpon*. Abundance of pathogenic strains of *Fusarium* or *Cylindrocarpon* within the styroblock containers is unknown.

## INTRODUCTION

Diseases caused by *Fusarium* spp. may cause serious losses to seedlings in northern Rocky Mountain nurseries (James 1986). Several recent investigations have indicated that inoculum of these fungi may reside and be transmitted from one crop of containerized seedlings to another within styroblock or pine Leach cell containers (James and Gilligan 1988; James, Gilligan and Reedy 1988). Losses from *Fusarium*-associated diseases have not been extensive at the Plum Creek Nursery in Pablo, Montana. However, an important disease of western white pine (*Pinus monticola* Dougl.) seedlings associated with *Cylindrocarpon* spp. has occurred periodically at the nursery (James 1987; James 1988).

Growers at the Plum Creek Nursery were concerned that potentially pathogenic fungi from the genera *Fusarium* and *Cylindrocarpon* may reside in high numbers within their styroblock containers which are reused for several seedling crops. They were unsure about the efficacy of their cleaning techniques in reducing amounts of these fungi within the containers. Therefore, an evaluation was conducted to determine background levels of styroblock contamination and to determine how fungal populations were influenced by their standard cleaning techniques.

LIBRARY COPY
ROCKY MTN. FOREST & RANGE
EXPERIMENT STATION

United States Department of Agriculture | Forest Service | Northern Region | P.O. Box 7669 Missoula, Montana 59807

## MATERIALS AND METHODS

Three Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco) seedlots (designated 631, 632, and 17045) were selected for analysis. Five styroblock trays containing seedlings which were several months old were randomly selected for sampling. Ten healthy-appearing seedlings per tray were selected and measured (height and caliper). Following seedling extraction, styroblock cells in which the selected seedlings had been growing were sampled. Two small (2-4 mm) pieces of styroblock were aseptically cut from the inner surface of cells at about 5 cm from the top and at the bottom of each cell. Pieces were placed inside surface down on a selective medium for *Fusarium* (Komada 1975). Plates were incubated at about 22 degrees C under cool fluorescent light for 5-7 days. Percentage of pieces colonized with *Fusarium*, *Cylindrocarpon*, and *Trichoderma* ( a common saprophytic fungus that may be antagonistic to *Fusarium*)Papavizas 1985) were calculated.

Styroblock containers were then cleaned using high-pressure steam. The same cells that had been sampled prior to cleaning were again sampled following cleaning. Two pieces at about 5 cm from the top and at the bottom of cells were extracted and incubated on Komada's medium as described above. Comparisons of fungal colonization between cleaned and uncleaned styroblock cells were made using standard "t" tests to evaluate efficacy of cleaning in reducing populations of fungi within cells. Simple linear regressions were used to determine extent of correlation between fungal colonization of cells and height of seedlings grown in those cells, i.e., are seedling heights (growth) adversely affected by fungi colonizing the inner surface of styroblock cells?

Several cells colonized with *Fusarium* were selected for further analysis. In these cells, pieces of styroblock were sampled at 7 and 10 cm from the top of cells. Four pieces were aseptically extracted (one in each of the four cardinal directions) and placed on Komada's medium and incubated as described previously. In this way, determination of the relative abundance of *Fusarium* and *Cylindrocarpon* at various locations within styroblock cells was obtained.

*Fusarium* isolates were transferred to potato dextrose agar (PDA) and carnation leaf agar and identified using the taxonomic guide of Nelson et al. (1983). Isolates of *Cylindrocarpon* were transferred to PDA and identified using criteria outlined by Booth (1966).

## RESULTS AND DISCUSSION

*Fusarium* spp. were isolated from almost 29 percent of the cells sampled (Table 1). This was generally less than was previously found at other nurseries (James and Gilligan 1988; James, Gilligan and Reedy 1988), but more than was previously isolated from a randomly selected container that had been fallow for several months at the Plum Creek Nursery (James 1987a). Much *Fusarium* inoculum was concentrated near the bottom of cells; amounts detected decreased toward the top of selected cells (Table 2). In several of the cells, *Fusarium* was only detected in the bottom. Steam cleaning of styroblocks significantly ($P=0.05$) reduced, but did not eliminate *Fusarium* detected within cells (Table 1). The cleaning technique used at the Plum Creek Nursery failed to remove many of the seedling roots that had grown into styroblock walls. These roots have been shown to harbor *Fusarium* (James, Gilligan and Reedy 1988) and may be important in carryover of inoculum from one crop to another.

Table 1.–Effects of cleaning on occurrence of Fusarium on styroblock containers at the Plum Creek Nursery, Pablo, Montana.

| | | Location within sytroblock cell | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Top 1/ | | | Bottom 1/ | | | Both 1/ | | |
| Seedlot | Cleaning | Cells 2/ | Pieces 3/ | Rating 4/ | Cells 2/ | Pieces 3/ | Rating 4/ | Cells 2/ | Pieces 3/ | Rating 4/ |
| 631 | Pre | 6.0 | 3.0 | 2.67 | 16.0 | 13.0 | 1.92 | 22.0 * | 8.0 * | 2.06 |
| | Post | 0 | 0 | 0 | 6.0 | 5.0 | 2.20 | 6.0 * | 2.5 * | 2.20 |
| 632 | Pre | 12.0 | 7.0 | 2.29 | 20.0 | 15.0 | 3.33 | 28.0 * | 11.0 * | 3.00 |
| | Post | 2.0 | 1.0 | 4.00 | 14.0 | 11.0 | 3.54 | 14.0 * | 6.0 * | 3.58 |
| 17045 | Pre | 6.0 | 3.0 | 2.00 | 34.0 | 26.0 | 2.61 | 36.0 * | 14.5 * | 2.55 |
| | Post | 2.0 | 1.0 | 1.00 | 16.0 | 12.0 | 2.92 | 16.0 * | 6.5 * | 2.77 |
| All Lots | Pre | 8.0 | 4.3 | 2.31 | 23.3 | 18.0 | 2.65 | 28.7 * | 11.2 * | 2.58 |
| | Post | 1.3 | 0.7 | 2.50 | 12.0 | 9.3 | 3.04 | 12.0 * | 5.0 * | 3.00 |

1/ Top = sample taken within styroblock container at about 5 cm from the top of each cell.
Bottom = sample taken at the bottom of each cell.
Both = a composite of both top and bottom samples.

2/ Percent of the sampled cells which were colonized with Fusarium.

3/ Percent of the styroblock pieces sampled (Two per cell at both top and bottom locations for pre- and post-cleaning samples) which were colonized with Fusarium.

4/ Average colonization intensity rating for pieces colonized (maximum = 4.00).

* Denotes significant differences (P = 0.05) between pre- and post-cleaning values using standard "t" tests. All percentages underwent arc-sin conversions prior to analyses.

Table 2.--Location of *Fusarium* and *Cylindrocarpon* within selected styroblock cells - Plum Creek Nursery, Pablo, Montana. *1/*

| Seedlot | No. cells sampled *2/* | Location from cell top (cm) | | | | Location from cell top (cm) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5 | 7 | 10 | 12.5 *3/* | 5 | 7 | 10 | 12.5 *3/* |
| 631 | 3 | 0 | 0 | 0 | 83.3 | 50.0 | 66.7 | 66.7 | 33.3 |
| 632 | 7 | 7.1 | 14.3 | 25.0 | 78.6 | 14.3 | 28.6 | 32.1 | 35.7 |
| 17045 | 8 | 6.2 | 9.4 | 25.0 | 75.0 | 0 | 43.7 | 46.9 | 18.7 |
| All lots | 18 | 5.6 | 9.7 | 20.8 | 77.8 | 13.9 | 41.7 | 44.4 | 27.8 |

*1/* Values in table are percent of styrofoam pieces sampled at the appropriate location that were colonized with either *Fusarium* or *Cylindrocarpon*.

*2/* Cells were selected on the basis of their being colonized at least to some extent by *Fusarium*.

*3/* Located at the bottom of cells.

*Cylindrocarpon* spp. were much more common within styroblocks than *Fusarium* spp. (Table 3). In randomly sampled cells, greater populations of *Cylindrocarpon* were detected at or near the bottom of cells. In cells selected specifically because they were colonized with *Fusarium*, *Cylindrocarpon* spp. were more concentrated in the middle of cells (Table 2). It is possible that these latter organisms were replaced with *Fusarium* in the bottom of cells. Although significant reductions in *Cylindrocarpon* populations occurred as a result of steam cleaning, relatively high levels persisted after cleaning. Since these fungi may be important in causing root decay of certain species (James 1987a; James 1988), persistance of high *Cylindrocarpon* levels in cleaned styroblocks may be important. On the other hand, many of these organisms may be saprophytic (Booth 1966) and of little concern. Pathogenicity evaluations are necessary to determine the potential problems that may result from high populations of *Cylindrocarpon* within styroblock containers.

Table 3.--Effects of cleaning on occurrence of Cylindrocarpon on styroblock containers at the Plum Creek Nursery, Pablo, Montana.

| | | Location within styroblock cell | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Seedlot | Cleaning | Top 1/ | | | Bottom 1/ | | | Both 1/ | | |
| | | Cells 2/ | Pieces 3/ | Rating 4/ | Cells 2/ | Pieces 3/ | Rating 4/ | Cells 2/ | Pieces 3/ | Rating 4/ |
| 631 | Pre | 94.0 | 81.0 | 3.20 | 96.0 | 91.0 | 3.52 | 100.0 NS | 86.0 NS | 3.37 |
| | Post | 82.0 | 74.0 | 3.32 | 94.0 | 87.0 | 3.79 | 98.0 NS | 80.5 NS | 3.24 |
| 632 | Pre | 40.0 | 32.0 | 2.47 | 82.0 | 76.0 | 3.43 | 84.0 * | 54.0 * | 3.15 |
| | Post | 26.0 | 23.0 | 2.87 | 74.0 | 63.0 | 3.65 | 74.0 * | 43.0 * | 3.44 |
| 17045 | Pre | 52.0 | 36.0 | 2.47 | 76.0 | 69.0 | 3.27 | 84.0 * | 52.5 * | 2.98 |
| | Post | 26.0 | 20.0 | 3.00 | 72.0 | 63.0 | 3.76 | 74.0 * | 41.5 * | 3.58 |
| All lots | Pre | 62.0 | 49.7 | 2.87 | 84.7 | 78.7 | 3.41 | 89.3 * | 64.2 * | 3.20 |
| | Post | 44.7 | 39.0 | 3.18 | 80.0 | 71.0 | 3.74 | 82.0 * | 55.0 * | 3.38 |

1/ TOP = sample taken within styroblock container at about 5 cm from the top of each cell; BOTTOM = sample taken at the bottom of each cell; BOTH = a composite of both top and bottom samples.

2/ Percent of the sampled cells which were colonized with Cylindrocarpon.

3/ Percent of the styroblock pieces sampled (Two per cell at both top and bottom locations for pre- and post-cleaning samples) which were colonized with Cylindrocarpon.

4/ Average colonization intensity rating for pieces colonized (maximum = 4.00).

* Denotes significant differences (P = 0.05); NS denotes nonsignificant differences between pre- and post-cleaning values using standard "t" tests. All percentages underwent arc-sin conversions prior to analyses.

Populations of *Trichoderma* were not concentrated in any portion of sampled cells and were not consistently reduced by steam treatments (Table 4). For example, in one seedlot (632) *Trichoderma* levels were reduced by cleaning. However, in another lot (17045) greater populations of these fungi were detected in cleaned styroblock cells. It also appeared that levels of *Trichoderma* detected within styroblocks were not inversely correlated with levels of *Fusarium* detected within the same cells as might be expected because of the antagonistic nature of *Trichoderma* (Papavizas 1985). Other fungi that were not detected because of use of the selective medium may have been responsible for the unpredictable occurrences of *Trichoderma* that were detected. From a standpoint of potential disease control, it would be preferable if high populations of *Trichoderma* were present in styroblocks and persisted despite cleaning at the expense of potential pathogenic fungi such as *Fusarium* and *Cylindrocarpon*. However, *Trichoderma* levels detected in this study were fairly low, especially compared to those of *Cylindrocarpon*. For greater biological control, it may be necessary to introduce higher levels of *Trichoderma* in the hope of reducing potential pathogens.

Table 4.--Effects of cleaning on occurrence of *Trichoderma* on styroblock containers at the Plum Creek Nursery, Pablo, Montana.

| | | Location within styroblock cell | | | | | |
|---|---|---|---|---|---|---|---|
| Seedlot | Cleaning | Top *1/* | | Bottom *1/* | | Both *1/* | |
| | | Cells *2/* | Pieces *3/* | Cells *2/* | Pieces *3/* | Cells *2/* | Pieces *3/* |
| 631 | Pre | 18.0 | 11.0 | 16.0 | 9.0 | 30.0 NS | 10.0 NS |
| | Post | 24.0 | 16.0 | 8.0 | 6.0 | 28.0 NS | 11.0 NS |
| 632 | Pre | 40.0 | 24.0 | 16.0 | 13.0 | 40.0 * | 18.5 NS |
| | Post | 12.0 | 9.0 | 22.0 | 15.0 | 26.0 * | 12.0 NS |
| 17045 | Pre | 24.0 | 15.0 | 24.0 | 17.0 | 36.0 * | 16.0 NS |
| | Post | 36.0 | 22.0 | 32.0 | 21.0 | 56.0 * | 21.5 NS |
| All lots | Pre | 27.3 | 16.7 | 18.7 | 13.0 | 35.3 NS | 14.8 NS |
| | Post | 24.0 | 15.7 | 20.7 | 14.0 | 36.7 NS | 14.8 NS |

*1/* Top = sample taken within styroblock container at about 5 cm from the top of each cell; Bottom = sample taken at the bottom of each cell; Both = a composite of both top and bottom samples.

*2/* Percent of the sampled cells which were colonized with *Trichoderma*.

*3/* Percent of the styroblock pieces sampled (Two per cell at both top and bottom for pre- and post-cleaning samples) which were colonized with *Trichoderma*. NS denotes nonsignificant differences between pre- and post-cleaning values using standard "t" tests. All percentages underwent arc-sin conversions prior to analyses. * denotes significant differences.

Correlations between amounts of *Fusarium* and *Cylindrocarpon* detected within styroblock cells and heights of seedlings growing within those cells were not high (Table 5). This would indicate that these fungi were apparently not important in limiting height growth of the sampled seedlings. The correlation between occurrence of *Fusarium* and *Cylindrocarpon* within the same cells was somewhat higher (Table 5), but still not very strong. Cells that had high levels of *Fusarium* tended to have lower levels of *Cylindrocarpon*, but other factors that were not tested may have been equally important. Antagonism between the two groups of organisms may not have occurred. However, colonization of the inner wall of styroblock cells to the exclusion of other organisms may have been more important.

Table 5.--Relationships between Douglas-fir seedling height and pre-cleaned styroblock cell colonization by *Fusarium* and *Cylindrocarpon* at the Plum Creek Nursery, Pablo, Montana.

| Comparison | Coefficient of Determination ($R^2$) | Regression equation |
|---|---|---|
| *1/* Percent cells<br>Seedling height (x) vs colonized with *Fusarium* (Y) | 0.06 | Y = -13.02 + 0.17 x |
| *1/* Percent cells<br>Seedling height (x) vs colonized with *Cylindrocarpon* (Y) | 0.06 | Y = 96.43 - 0.24 x |
| Percent cells Percent cells<br>colonized with *Fusarium* (x) vs colonized with *Cylindrocarpon* (Y) | 0.26 | Y = 66.18 - 0.70 x |

*1/* Average seedling heights for the three seedlots were:

| Seedlot | Average height (mm) |
|---|---|
| 631 | 167.6 |
| 632 | 165.1 |
| 17045 | 180.2 |

The most commonly isolated species of *Fusarium* was *F. oxysporum* Schlect., which was frequently isolated from both cleaned and uncleaned cells. The other species isolated were *F. sambucinum* Fuckel and *F. acuminatum* Ell. & Ev. Although these species were found less frequently than *F. oxysporum*, they were still common. It is not known which of these organisms were pathogens. From previous tests (James et al. 1988), isolates of both *F. oxysporum* and *F. acuminatum* may be pathogenic or saprophytic, whereas those of *F. sambucinum* are generally saprophytic. There is currently no easy way to quickly ascertain whether isolates of *Fusarium* are pathogenic without conducting inoculation tests on seedlings. However, it is possible that isozyme and/or genetic analyses may be useful in catagorizing isolates quickly. For the present, we can probably conclude that some of the isolates obtained from styroblock containers are pathogens and others are not.

Two species of *Cylindrocarpon* were isolated from styroblock containers. The most common was *C. didymum* (Hartig) Wollew., the principle species isolated from the roots of western white pine seedlings at the nursery (James 1987b; James 1988). The other species was *C. tenue* Bugn. Both of these species may be important components of the rhizosphere of plants (Andrews and Clouston 1937; Booth 1966) and may be associated with diseases of conifer seedlings (Houten 1939; James 1987b; James 1988; James and Gilligan 1985). However, isolates of both may be saprophytic as well (Booth 1966). Therefore, like the fusaria, some of these isolates of *Cylindrocarpon* may be important in the development of seedling diseases at the nursery while others are not.

Problems of adequately cleaning styroblock containers exist for many growers. Existing techniques of using high pressure steam and/or cleaning solvents do not appear to be very effective. Even dipping blocks in a chlorine bleach solution does not adequately reduced levels of *Fusarium* (James, Gilligan and Reedy 1988). One grower even used gaseous methyl bromide to try to eliminate these organisms and was unsuccessful (R. Schaeffer, personal communication). However, several growers in Canada have had good results in sterilizing styroblocks with sodium metabisulfite (R. Sturrock, personal communication). This chemical gives off sodium dioxide when mixed with water which effectively fumigates blocks and kills nearly all organisms, including *Fusarium*. However, potential latent phytotoxic effects have not yet been evaluated and should be tested prior to widespread use. Efficacy of this chemical on other types of seedling containers, such as pine Leach cells is unknown.

## LITERATURE CITED


Andrews, F. W. and T. W. Clouston. 1937. Section of Botany and Plant Pathology. Rep. Dep. Agric. Forest., Sudan Part 2:32.

Booth, C. 1966. The genus *Cylindrocarpon*. Mycological Papers No. 104. Commonwealth Mycological Institute, Kew, Surrey, England. 56p.

Houten, J. G. 1939. Kiemplantenziekten von coniferen. Thesis, University of Utrecht. 125p.

James, R. L. 1986. Diseases of conifer seedlings caused by seedborne *Fusarium* species. *In*: Shearer, R. C. (compiler). Proceedings: Conifer Tree Seed in the Inland Mountain West Symposium. USDA Forest Service, Intermountain Res. Sta., Gen. Tech. Rept. INT-203. pp. 261-271.

James, R. L. 1987a. Occurrence of *Fusarium* within styroblock containers-Plum Creek Nursery, Pablo, Montana (Preliminary Report). USDA Forest Service, Northern Region. Nursery Disease Notes #51. 2p.

James, R. L. 1987b. Root deterioration of containerized western white pine seedlings-Plum Creek Nursery, Pablo, Montana. USDA Forest Service, Northern Region. Nursery Disease Notes #47. 5p.

James, R. L. 1988. Root disease of western white pine seedlings-Plum Creek Nursery, Pablo, Montana. USDA Forest Service, Northern Region (In preparation).

James, R. L., R. K. Dumroese, C. J. Gilligan, and D. L. Wenny. 1988. Epidemiology of *Fusarium* on containerized Douglas-fir seedlings. 3. Pathogenicity of selected *Fusarium* isolates from Douglas-fir seed and seedlings. USDA Forest Service, Northern Region (In preparation).

James, R. L. and C. J. Gilligan. 1985. Containerized Engelmann spruce seedling diseases at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region. Rept. 85-17. 15p.

James, R. L. and C. J. Gilligan. 1988. Occurrence of *Fusarium* on Leach pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region Rept. 88-8.

James, R. L., C. J. Gilligan and V. Reedy. 1988. Evaluation of root diseases of containerized conifer seedlings at the Champion Timberlands Nursery, Plains, Montana. USDA Forest Service, Northern Region (In press).

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Protec. Res. 8:114-125.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press. University Park. 193p.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Ann. Rev. Phytopathol. 23:23-54.